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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Stephen J. Garger, *et al.*

Application Serial No. 09/626,127

Filed: July 26, 2000

Art Unit: 1656

Examiner: J. Riley

Attorney Docket No. 00801.0087.CPUS04

For: **PRODUCTION OF LYSOSOMAL ENZYMES IN PLANTS BY TRANSIENT EXPRESSION**

DISK TO STIC

RESPONSE TO FINAL OFFICE ACTI(

DATE:

OK TO ENTER 4/30/02

BOX AF
Commissioner for Patents
Washington, D.C. 20231

Sir:

This is a Response to the Office Action dated November 29, 2001. The response is submitted prior to or including the extended due date of **March 29, 2002.** A petition for a one-month extension of time and the requisite fee are enclosed herewith. The Examiner is respectfully requested to enter the amendments and reconsider the application.

THE AMENDMENT

In the Specification

The paragraph beginning at page 12, line 36 is as follows:

D1 "FIGURE 2 shows a Tobamovirus expression vector containing the human α galactosidase gene or a variant of the gene (includes SEQ ID NOs: 1-3)."

The paragraph beginning at page 13, line 9 is as follows:

"FIGURE 5 shows carboxy terminal modifications to α galactosidase (SEQ ID NOs:3-12)."

"FIGURE 14 shows transgenic vector pBSG638 for rGCB expression.

FIGURE 15 shows viral vector pBSG641 for rGCB expression."

The paragraph beginning at page 13, line 21 is as follows:

"FIGURE 12 shows TTODA (rGAL-12R) TMV RNA begins at base 1; 126/183 reading frame begins at 69, 3417 is suppressible stop codon, and ends at 4919.30K ORF begins at 4903 and ends at 5709. Human α galactosidase A RNA begins at 5703, α amylase signal peptide is from 5762-5857; mature human α galactosidase A coding region is 5858-7036, ToMV virus coat protein and 3 UTR follows (SEQ ID NO:13)."

The paragraph beginning at page 13, line 26 is as follows:

"FIGURE 13 shows SBS5-rGAL-12R TMV RNA begins at base 1; 126/183 reading frame begins at 69, 3417 is suppressible stop codon, and ends at 4919.30K ORF begins at 4903 and ends at 5709. Human α galactosidase A RNA begins at 5703, complete (signal peptide and mature protein coding region) human α galactosidase A gene 5766-7037, TMV U5 virus coat protein and 3 UTR follows (SEQ ID NO:14)."

The paragraph beginning at page 22, line 3 is as follows:

"Transgenic Tobacco Leaves Express Moderate Levels of rGCB. We combined a dual promoter from Cauliflower Mosaic Virus (35S), a translational enhancer from Tobacco Etch Virus and a polyadenylation region from the nopaline synthetase gene of *Agrobacterium tumefaciens* with the native human GCB cDNA to create plasmid pBSG638 (33; Fig. 14). These expression elements are widely used to provide the highest possible constitutive expression of nuclear encoded genes. Depending on the nature of individual proteins, these vectors can be used to accumulate moderate levels of recombinant proteins in most tissues of many plant species."

The drawing on page 22 between lines 11-15 is deleted, and submitted as FIGURE 14.

STRUCTURE OF THE N-TERMINUS OF rGCB

N-terminal Amino Acid Sequence	
X X P X I P K S F G Y	rGCB from tobacco (SEQ ID NO:15)
A R P C I P K S F G Y	GCR human (SEQ ID NO:16)

The drawing on page 30 between lines 1-4 is deleted, and submitted as FIGURE 15.

The paragraph beginning at page 30, line 5 is as follows:

"We introduced this construct (Fig. 15) into Agrobacterium and transformed tobacco plants as described above. In this case many of the plant leaves displayed necrotic lesions as transfection events randomly occurred during growth and development and expansion of leaves. These lesions never formed on control transformed plant lines containing vector only sequences capable of replication. These lesions were identical in appearance to the types of lesions induced by plant pathogens during a type of disease resistance reaction, termed the hypersensitive response (HR). Therefore, under conditions where we expect to accumulate large quantities of active enzyme, an HR is signaled by some component of the vector infection specific to rGCB. There are very few of these so-called HR "elicitors" characterized in the literature. Possibly the rGCB enzyme itself, or a secondary metabolite resulting from enzymatic activity, or even rGCB RNA, may induce the HR. In any case, we hypothesize that the HR selects for loss of the gene from the viral RNA population. It is important to remember that this is not a simple genetic instability phenomenon. Under conditions where an HR is not induced, we have synthesized many proteins using TMV-based RNA viral vectors to levels of several percent of the total soluble cell protein without loss of the inserted

The paragraph beginning at page 47, line 1 is as follows:

"In order to ensure efficient delivery of rGal-A into the lumen of the plant endoplasmic reticulum, we fused the Gal-A cDNA (31) to a plant signal peptide sequence derived from rice α-amylase gene (32,33). We also hypothesized that addition of an ER-retention signal (SEKDEL) (SEQ ID NO:18) might prolong the resident time of the recombinant protein in the ER to increase the fraction of correctly assembled and catalytically active enzyme under extreme conditions of protein synthesis. These constructs were subcloned into the viral vector TTODA, a chimera between tobacco and tomato mosaic viruses (Fig. 1). Transcripts were prepared in vitro and inoculated onto the lower leaves of whole plants (Nicotiana benthamiana). 1-3 weeks after inoculation, leaves were weighed, rolled in a strip of Parafilm and placed in a disposable chromatography column and submerged in enzyme extraction buffer (0.1 M K/P04, 0.1 M NaCl, 5 mM EDTA, 10 mM β-ME and 0.5% sodium taurocholate, pH 6.0). In order to infiltrate the buffer into the tissue, a vacuum of 730-750 mmHg was twice applied. After draining the excess buffer, the intercellular fluid fraction was recovered by low-speed centrifugation (~1,500 X g, 15 min). To measure enzyme remaining in the tissue after this treatment, the leaf was unrolled after centrifugation and two discs removed with a #14 cork borer. This tissue sample was transferred to an eppendorf tube, frozen in liquid nitrogen and ground in four volumes of enzyme extraction buffer. In rGal-A enzyme assays, we measured cleavage of the fluorogenic substrate 4-methyl umbelliferyl α-D-galactopyranoside (4-MUG) against known standards using established protocols (34). Units are nmoles of 4-MUG hydrolyzed per hour at 37°C."

The table beginning at page 50, line 1 is amended as follows:

" Table 8. "

Vector Designation	Carboxy-Terminal Modifications (Amino Acid Sequence)
rGAL-A	TSRLRSHINPTGTVLLQLENTMQMSLKDLL (SEQ ID NO:3)
rGAL-AR	TSRLRSHINPTGTVLLQLENTMQMSLKDLLSEKDEL (SEQ ID NO:4)
rGAL-4	TSRLRSHINPTGTVLLQLENTMQMSL (SEQ ID NO:5)
rGAL-4R	TSRLRSHINPTGTVLLQLENTMQMSLSEKDEL (SEQ ID NO:6)
rGAL-8	TSRLRSHINPTGTVLLQLENTM (SEQ ID NO:7)
rGAL-8R	TSRLRSHINPTGTVLLQLENTMSEKDEL (SEQ ID NO:8)
rGAL-12	TSRLRSHINPTGTVLLQL (SEQ ID NO:9)
rGAL-12R	TSRLRSHINPTGTVLLQLSEKDEL (SEQ ID NO:10)
rGAL-25	TSRLR (SEQ ID NO:11)
rGAL-25R	TSRLRSEKDEL (SEQ ID NO:12)

In the Claims

Cancel Claim 1

The Amendments